47 LAMOTRIGINE QUANTITATION AND CONFIRMATION BY LCMS

47.1 Summary

47.1.1 Lamotrigine is extracted from biological samples with an acetonitrile precipitation and analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).

47.2 Specimen Requirements

47.2.1 0.2 mL blood, serum or plasma.

47.3 Reagents and Standards

47.3.1 Ammonium Formate

47.3.2 Methanol

47.3.3 Acetonitrile

47.3.4 Lamotrigine (Lamictal®)

47.3.5 Phenacetin

47.4 Solutions, Internal Standards, Calibrators and Controls

47.4.1 10 mM Ammonium formate with acetonitrile: Weigh 0.315 g ammonium formate. Transfer to 500 mL volumetric flask and QS to volume with dH_2O. Then add 25 mL acetonitrile.

47.4.2 Working standard solutions for lamotrigine

47.4.2.1 1 mg/mL lamotrigine stock solution: Weigh 10mg of lamotrigine. Transfer to 10 mL volumetric flask and QS to volume with methanol.

47.4.2.2 0.02 mg/mL lamotrigine working solution: Pipet 200 µL of 1.0 mg/mL stock solution of lamotrigine into 10.0 mL volumetric flask and QS to volume with methanol.

47.4.3 Quality Control (QC) standard solutions

47.4.3.1 0.02 mg/mL lamotrigine QC solution: Pipet 200 µL of separate 1.0 mg/mL stock solution of lamotrigine into 1.0 mL volumetric flask and QS to volume with methanol.

47.4.4 Internal standard working solution

47.4.4.1 1 mg/mL phenacetin stock solution. Weigh 10 mg phenacetin, transfer to 10 mL volumetric flask and QS to volume with methanol.

47.4.4.2 0.1 mg/mL phenacetin: Pipet 1 mL of 1 mg/mL phenacetin stock solution into 10 mL volumetric flask and QS to volume with methanol

47.4.5 Calibrators. To prepare the calibration curve, pipet the following volumes of working solution into appropriately labeled 16 x 125 mm screw cap tubes and take to dryness:

47.4.5.1 Cal 1: 12 mg/L lamotrigine: 120 µL of 0.02 mg/mL lamotrigine working solution

47.4.5.2 Cal 2: 10 mg/L lamotrigine: 100 µL of 0.02 mg/mL lamotrigine working solution

47.4.5.3 Cal 3: 8 mg/L lamotrigine: 80 µL of 0.02 mg/mL lamotrigine working solution

47.4.5.4 Cal 4: 4 mg/L lamotrigine: 40 µL of 0.02 mg/mL lamotrigine working solution

47.4.5.5 Cal 5: 2 mg/L lamotrigine: 20 µL of 0.02 mg/mL lamotrigine working solution

47.4.5.6 LOD: 1 mg/L lamotrigine: 10 µL of 0.02 mg/mL lamotrigine working solution

47.4.5.7 For each calibrator, add .2 mL blank blood to each tube to achieve final concentration.

47.4.6 Lamotrigine Control (QC)

47.4.6.1 5 mg/L Lamotrigine: Pipet 50 µL of 0.02 mg/mL lamotrigine QC solution into appropriately labeled 16 x 125 mm screw cap tube and add .2 mL blank blood.

47.4.6.2 Negative blood control: Blood bank blood (or equivalent) previously determined not to contain lamotrigine.

47.5 Apparatus

47.5.1 Screw cap test tubes, 16 x 125 mm

47.5.2 Centrifuge capable of 2,000-3,000 rpm

47.5.3 Vortex mixer

47.5.4 GC autosampler vials with inserts

47.5.5 LC/MS: Agilent Model 1100 LC-MSD

47.5.5.1 LCMS Instrument Conditions. The following instrument conditions may be modified or adjusted to improve separation and sensitivity. Quantitation can be achieved by either collecting data in SIM or Scan mode.

47.5.5.1.1 Elution conditions:

47.5.5.1.1.1 Column: Altima HP HILIC 150 mm X 2.1 mm, 3 µm particle size
47.5.5.1.1.2 Column thermostat: 35° C
47.5.5.1.1.3 Solvent A: 10 mM ammonium formate in dH_2O containing acetonitrile
47.5.5.1.1.4 Solvent B: Acetonitrile
47.5.5.1.1.5 Solvent ramp stop time: 10 min

Time	Solv. A	Solv. B	Flow
Initial	5%	95%	.700
4.00	11%	89%	0.400
5.00	5%	95%	0.700

47.5.5.1.2 Spray Chamber

47.5.5.1.2.1 Ionization Mode: Electrospray
47.5.5.1.2.2 Gas Temperature:350° C
47.5.5.1.2.3 Drying Gas (N_2): 12.0 L/min
47.5.5.1.2.4 Nebulizer pressure: 30 psig
47.5.5.1.2.5 Vcap (Positive): 4000 V
47.5.5.1.2.6 Injection Volume: 1µL

47.5.5.1.3 Selected Ion Monitoring

Time	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0.00 min	Phenacetin	180	150	1	Low	108
	Lamotrigine	256	150	1	Low	108
	Lamotrigine	257	150	1	Low	108
	Lamotrigine	258	150	1	Low	108
	Lamotrigine	259	150	1	Low	108

47.5.5.1.3.1 Polarity: Positive
47.5.5.1.3.2 SIM parameters (quantitation ion)

47.5.5.1.4 Scan Monitoring

Time (min)	Low Mass	High Mass	Fragmentor	Gain EMV	Threshold	Stepsize
0.00	55.0	300.0	150	1.0	150	0.10

47.6 Procedure

47.6.1 Label 16 x 125 mm screw cap tubes appropriately with blank, calibrators, controls and case sample IDs.

47.6.2 Prepare calibrators and controls.

47.6.3 Add .2 mL case specimens to the appropriately labeled tubes.

47.6.4 Add 50 µL 0.1 mg/mL phenacetin internal standard working solution to each tube.

47.6.5 Slowly, add dropwise 6 mL acetonitrile to each tube while vortexing. Continuous vortexing, not mere mixing, is essential.

47.6.6 Vortex an additional 30 seconds.

47.6.7 Centrifuge at approximately 2800 rpm for 15 minutes.

47.6.8 Transfer an aliquot of clear acetonitrile mixture to a GC vial containing a conical insert and transfer to autosampler tray.

47.6.9 Inject 1 µL of each sample on LC/MS in the API-ES/SIM or Scan Mode.

47.7 Calculation

47.7.1 Drug concentrations are calculated by linear regression analysis using the ChemStation software.

47.8 Quality Control

47.8.1 Limit of Detection (LOD) = 1 mg/L.
47.8.2 Limit of Quantitation (LOQ) = 2 mg/L.
47.8.3 Upper limit of linearity (ULOL) = 12 mg/L
47.8.3.1 Samples greater than the ULOL should be reported as "greater than 12 mg/L" if not re-analyzed with a dilution.
47.8.3.2 Blank calibration matrix (whole blood, serum, or plasma) **must be** used as the diluent. Samples **will not be diluted** with distilled water.
47.8.4 Re-injection of processed samples **is not permitted**. Samples will be re-processed for analysis.
47.8.5 See Toxicology Quality Guidelines for additional parameters.

47.9 References

47.9.1 Dwight D. Flammia Ph.D., and Les Edinboro Ph.D. In house development.

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